

## Molecular Weights of Humic Acids in Sulfolane

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### INTRODUCTION

Recent viscosimetric studies by Mukherjee and Lahiri (1) and by Rajalakshmi et al. (2) offer convincing evidence that humic acids behave as polyelectrolytes. Since this behaviour is fundamentally inconsistent with previously reported molecular weights - which range from 160 to 700 when measured cryoscopically in catechol or acetamide (3, 4) - re-examination of the molecular weight of humic acids appears to be fully warranted.

The difficulties inherent in such a study are formidable and have been discussed by several investigators (1, 2, 3, 5, 6, 7). For example, Yokokawa (7) notes that molecular weight determinations on solutions of humic acids in acetamide amount to little more than an estimate of the proportion of functional groups. From conductivity measurements and parallel cryoscopic determinations on potassium humates in aqueous solutions, he concluded that some of his preparations had molecular weights in the thousands rather than in the hundreds. Osmotic pressure techniques (8) have likewise yielded molecular weights estimated in the range of 1200-2200. And Dryden (9), using semi-quantitative diffusion measurements, concluded that some 90% of the ethylene diamine extract of coal\* has a molecular weight in excess of 6000.

Some of the specific problems associated with cryoscopic measurements on humic acids emerge from an analysis of published data, e.g. those of Polansky and Kinney (4). A particularly noteworthy feature of these data is the variation of the cryoscopic constant  $K$  with solute concentration, which suggests some form of molecular association, dissociation or solvation in acetamide. Fig. 1, which reproduces typical results obtained in this laboratory with 1, 2, 4, 5 - benzene tetracarboxylic acid in acetamide, may serve to illustrate the general effect: the curvilinear shape of the graph suggests complex behaviour including probable ionization at low molality and solvation at higher molalities (10). A second difficulty to be overcome in molecular weight determinations by cryoscopic means relates to the removal of water without simultaneous chemical change and loss of solubility. Finally, it should be recognized that humic acid preparations, particularly those obtained by oxidative degradation of coal, may show a wide distribution of molecular weights. In order to attach real significance to a number-average molecular weight of such materials, and permit a meaningful comparison of  $M_n$  with weight-average molecular weights obtained by other methods, it is, therefore, important to undertake appropriate fractionation of the sample.

To a considerable degree, however, it appears that most of these difficulties can now be eliminated. Burwell and Langford (11) recently suggested that tetramethylene sulfone (sulfolane) because of its low latent heat of fusion and resulting high cryoscopic constant ( $K$ ) might represent a good medium for the cryoscopic determination of molecular weights, and this suggestion seems to have real merit. It has been found that sulfolane is not only a reasonably good solvent for humic acids, but that it behaves as an essentially ideal solvent for a variety of solids with normally quite distinct associative and dissociative tendencies. The detailed findings are reported below.

\*While humic acids need not necessarily have similar molecular weights, some preparations or fractions of humic acids prepared from coal might be expected to lie in such a range.

## EXPERIMENTAL

### Materials

Acetamide used was Fisher certified reagent dried in an oven at 60°C/20 mm. for 24 hours and stored in a desiccator over phosphorous pentoxide until used.

Sulfolane was the product of Shell Development Co., Emeryville, California. This material was distilled from powdered sodium hydroxide under reduced pressure and the portion boiling at about 100°C/0.01 mm. retained for use.

1, 2, 4, 5 - Benzene tetracarboxylic acid (mp. 177-8°C) was obtained from Aldrich Chemical Co., dried and stored in the same manner as the acetamide.

$\beta$ -Naphthalene sulfonic acid was recrystallized from an alcohol-benzene mixture as the hydrate, but no attempt was made to dry it as this was accomplished in the course of its use (see below).

Humic acids were derived from two sources. One was commercially available Baroid Carbonox, a naturally oxidized (i.e. weathered) North Dakota Lignite, and the other a weathered sub-bituminous coal from the upper seam at Sheerness, Alberta. Each was extracted with sodium hydroxide solution, filtered, precipitated with hydrochloric acid, washed until nearly free of salts, and finally electrodialyzed until the current had fallen to a minimum.

### Cryoscopic Procedure

Cryoscopic measurements were carried out in an apparatus similar to that employed by Smith and Howard (3) and Polansky and Kinney (4) and flooded with purified nitrogen to exclude moisture. Measurements in acetamide were performed following the procedure of Polansky and Kinney (4). When sulfolane (mp. 28.2°C) was used, the bath was slightly cooled by a cold water-coil in order to permit operation of the heater-controller.

Materials such as biphenyl and benzoic acid, which did not present a significant drying problem, were weighed out and directly dissolved in sulfolane for cryoscopic constant determinations. In the case of  $\beta$ -naphthalene sulfonic acid, it was found more convenient to dissolve a roughly weighed sample in more sulfolane than actually required and subsequently to distil off part of the solvent (and all water) at 100°C/0.01 mm. The acid concentration was then determined by titrating an aliquot of the solution with standard alkali.

### Molecular Weight Determinations

Since humic acids did not dissolve rapidly in sulfolane, and since it was also necessary to use a drying technique on the resultant solution, it was found most convenient to dissolve the sample (300-500 mg.) in 20-30 ml. of a 3:1 acetone-water mixture, add this to 55-60 ml. sulfolane, filter the mixture repeatedly (5-8 times) through a Seitz bacterial (sterilizing) filter (previously found adequate to render the filtrates optically void) and remove the acetone, water and some sulfolane under reduced pressure (with the final conditions not exceeding 100°C/0.01 mm.). After determination of the freezing point depression, the weighed sulfolane solution was diluted with sufficient benzene to precipitate the humic acid, which was then removed by centrifugation, washed free of sulfolane with more benzene, dried and weighed. In the case of lower molecular weight humic acids, a significant amount of material remained in solution in the mother liquor after the first precipitation; this was recovered by concentrating the solution to a very small

volume under reduced pressure and further dilution with benzene. The precipitate was then treated like the first one and added to it.

The techniques described above for sample drying eliminate the water problem entirely. Because of the large difference in the boiling points of sulfolane ( $278^{\circ}\text{C}/700\text{ mm.}$ ;  $100^{\circ}\text{C}/0.01\text{ mm.}$ ), and water it was possible to remove water entirely by vacuum distillation prior to the freezing point determination. Caution is, however, necessary in the case of humic acid solutions, whose exposure to temperatures much in excess of  $100^{\circ}\text{C}$  results in the formation of anhydrides with consequent changes in solubility. This aspect will be discussed in another paper.

#### The Cryoscopic Constant of Sulfolane

The average cryoscopic constant of sulfolane ( $K = 65.5^{\circ}\text{C}/\text{mole}/\text{kg.}$ ), which agreed well with the figure of  $66.2 \pm 0.6^{\circ}\text{C}/\text{mole}/\text{kg.}$  quoted by Burwell and Langford (11), was established by measuring the freezing point depression ( $\Delta T$ ) for various concentrations of benzoic acid, biphenyl and  $\beta$ -naphthalene sulfonic acid. The results are shown in Fig. 2 and indicate that the constant is valid for use with materials of vastly differing dissociative and associative potentialities.

#### Fractionation

Humic acids were prepared for molecular weight determination by two fractionation techniques, one involving successive extractions with an alcohol-benzene mixture and the other precipitation from sulfolane solution with benzene as the non-solvent. In the former case, 14.18 g. of Carbonox humic acid (average m.w. 1570) was extracted in a soxhlet apparatus with the azeotrope of alcohol and benzene for 25 days; the results are set out in Table I. Infrared spectra were obtained for each fraction, typical ones being shown in Fig. 3. Apart from small variations in the carbonyl band, which are listed in Table I, all spectra were similar.

In the second method, a 10 g. sample of Sheerness, Alberta, humic acid (average m.w. 4600) was dissolved in 200 ml. of sulfolane by the acetone-water technique described above, filtered through a Seitz (sterilizing) filter until optically void and then diluted with 130 ml. of benzene. The resultant precipitate was removed in the centrifuge, washed with benzene and dried at  $60^{\circ}\text{C}/20\text{ mm.}$

The remaining solution was treated with more benzene to produce a second precipitate and so on. The general procedure is summarized in Table II.

#### DISCUSSION

A number of factors combine to make sulfolane a peculiarly attractive solvent for cryoscopic measurements. Its cryoscopic constant (65.5 degrees per mole) is very much higher than those of other readily available solvents (thus permitting accurate measurements of freezing point depressions at low solute concentrations). It has good solvent properties for humic acids and a wide variety of simpler materials. It permits effective drying of hygroscopic materials and also allows their concentration to be determined quantitatively after drying. And most important, sulfolane appears to eliminate disturbing effects arising from association or dissociation of solute molecules.

Fig. 2 supports this last contention by showing that sulfolane exhibits ideal cryoscopic behaviour - cf. Spauschus (10) - with  $K$  values independent of concentration for benzoic acid,  $\beta$ -naphthalene sulfonic acid and biphenyl. It should be emphasized that this constancy of  $K$

Table I  
Fractionation of Carbonox Humic Acid with Alcohol-Benzene  
(Average molecular weight of starting material 1570)

Fraction	Incremental Extraction Time	Extract (% of original sample)	%C*	%H	E <sup>0.4%</sup> ** 0.09 cm.	Molecular Weight
1	2 hours	8.21			19.4	600
2	3 hours	6.77	56.98	4.39	23.9	970
3	12 hours	24.75	57.46	4.26	21.0	1546
4	48 hours	6.19	58.30	4.30	18.2	
5	12 hours	1.61	57.84	3.95		
6	24 hours	1.01	57.32	4.40	18.3	
7	24 hours	0.95	57.76	4.19	19.9	
8	4 days	5.39	58.40	4.02	18.3	5370
9	7 days	1.07	58.96	4.04	19.1	
10	7 days	1.01	58.78	4.15	19.3	
Residue		45.46	57.91	3.19	13.4	
Total		102.42				

\*

Carbon and hydrogen analyses were performed by Micro-Tech Laboratories, 8000 Lincoln Avenue, Skokie, Illinois.

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E<sup>0.4%</sup><sub>0.09 cm.</sub> = extinction coefficient of carbonyl band (1720 cm.<sup>-1</sup>)  
(determined in KBr)

Table II  
Fractionation of Sheerness Humic Acids by Precipitation from Sulfolane (200 ml.)  
(Average molecular weight of starting material 4600)

Fraction	Incremental Volume of Benzene added	Precipitate (% of original sample)	%C	%H	Molecular Weight
1	130	66.13			See note A
2	100	9.98	56.8	4.58	
3	100	3.77	58.6	4.43	1, 260
4	400	0.64	57.95	4.90	
5	material remaining in solution	2.21	59.37	4.66	460
Total		82.72 *			

\*

A material balance shows that some 17% of this initial sample were lost during fractionation. We are inclined to the view that this loss accrues from partial peptization of this humic acid and elimination of the peptized material with the washings.

Note A

Fraction 1 was dissolved in 200 ml. of sulfolane and 100 ml. benzene added. The fraction which precipitated (3.55 g.) had molecular weight 8,500, and contained 52.1% C, 4.00% H.

would not be observed if the carboxylic or sulfonic acid groups were to ionize or associate in sulfolane solution. Burwell and Langford (11) cite three other examples of this ideality in sulfolane solution, the most interesting from the standpoint of the present study being acetic acid, which they describe as behaving as a monomer when dissolved in sulfolane.

That sulfolane solutions of humic acid also yield concentration-independent results is illustrated by the fact that the observed molecular weights of a humic acid preparation over the 0.01-0.005 molal range varied only between 1520 and 1560. This variation is considered to lie within the normal experimental error incurred in the measurement.

On the assumption that association would be at a maximum in the solid state and at a minimum in dilute solution, further evidence for the absence of association in sulfolane solution can be derived from infrared data, in particular from analysis of the carbonyl stretching frequency between 1733 and 1695  $\text{cm}^{-1}$  and from the -OH stretching frequency between 3700 and 2500  $\text{cm}^{-1}$ .

When benzoic acid is examined in a Nujol mull, the carbonyl absorption band appears at 1695  $\text{cm}^{-1}$ , while a 0.5% solution in sulfolane shows this band at 1733  $\text{cm}^{-1}$ . Since such a shift is known to be associated with a change from the dimeric to the monomeric form of the acid (12), molecular association in sulfolane solution would imply a progressive shift of the carbonyl band towards 1695  $\text{cm}^{-1}$  or the appearance of a second band there (12) as the solute concentration increases. This does not occur: even for a 5% solution of benzoic acid in sulfolane, the carbonyl absorption was found to remain at 1733  $\text{cm}^{-1}$ .

The same conclusion can be derived from the position of the -OH stretching band. According to Bellamy (13) and Flett (12) absorption between 2700 and 2500  $\text{cm}^{-1}$  is commonly taken as evidence for the existence of dimeric carboxylic acid groups. This is observed in the solid state spectrum of benzoic acid, but is absent in spectra of sulfolane solutions. The -OH band which did appear occurred at 3226  $\text{cm}^{-1}$ . This is too low for completely free -OH (3700-3500  $\text{cm}^{-1}$ ) but is in the region of weak hydrogen bonding. Since the cryoscopic results showed no variation with concentration, and since the carbonyl absorption showed no indication of dimerization of the benzoic acid molecules, this shift does not appear to be caused by inter-action between the acid molecules. It is possible that the -OH group is hydrogen-bonded to an oxygen atom of the sulfolane, cf. Jones (14). This would remove some of the solvent molecules from that role and make them in effect part of the solute. However, over the concentration range here studied this has been calculated to exert a negligible influence on the effective concentration.

The unsuitability of acetamide as a cryoscopic solvent is borne out by the variation of its cryoscopic constant (determined with 1, 2, 4, 5 - benzene tetracarboxylic acid solute) with solute concentration (Fig. 1). Spauschus (10) reported similar behaviour for high molecular weight esters in benzene and discussed the large departure from ideality in the region of low concentration. Assuming that a similar behaviour to that seen here for benzene tetracarboxylic acid would be exhibited by humic acids in acetamide solution, it is likely that previously reported measurements were carried out in the region of large deviations from ideality (below 0.03 molal), since the materials used probably had number average molecular weights in excess of 1000.

Further evidence for the unsuitability of acetamide for cryoscopic work is offered by Magne and Skau (15) who note that the molar depressions of closely related fatty acids in acetamide vary widely. They suggest that differing degrees of dissociation of the molar complexes of these materials with acetamide may explain this phenomenon.

Considerable errors would also be introduced if insufficiently dried humic acids were used. For example, 1% water in a material of m.w. 1500 would double  $\Delta T$ . The same impurity would result in a 3-fold increase in  $\Delta T$  if the molecular weight were 5000.

In order to show that the humic acids here studied were also subject to these uncertainties in acetamide, a sample of molecular weight 970 (determined in sulfolane) was found to give a value of 230 in acetamide (using  $K = 3.58$ ).

The fractionation procedures described above provided samples which, from several stand-points, are more appropriate for molecular weight studies than an aggregate, unfractionated sample. For example, in order to compare values obtained by number and weight average techniques, as small a range of molecular sizes as possible is desirable.

There is reason to believe that further fractionation is possible and that the molecular weights of humic acids may extend well beyond the upper limit reported here. The method adopted in this study is limited by the fact that acids of high equivalent weight and/or very high molecular weight are difficultly soluble in sulfolane.

In the case of fractionation by extraction with an alcohol-benzene mixture, attempts to determine the molecular weight of the residue encountered difficulties. The sample would only incompletely dissolve in either acetone-water or in this mixture plus sulfolane, and each attempted molecular weight determination accordingly resulted in different  $\Delta T$ 's with calculated molecular weights ranging from 985 to 1510. We venture to suggest that this behaviour is intimately connected with particular properties of the residue. While a residue fraction with an apparent molecular weight of 985 was shown by sodium aminoethoxide titration to have an equivalent weight of 240, previously separated alcohol-benzene soluble fractions, irrespective of their molecular weights, had equivalent weights of about 140. It is, therefore, reasoned that the residual material includes both high and low molecular weight fractions characterized by a relative lack of polarity. This view is consistent with the low carbonyl extinction coefficient of  $E_{0.09}^{0.4\%} = 13.4$  for the whole residue as compared with an average of

$E_{0.09}^{0.4\%} = 19.6$  for the soluble fractions.

It has thus been shown that the molecular weights of humic acids dissolved in sulfolane, and thoroughly freed from water, can be determined satisfactorily by the cryoscopic method. The resultant measurements appear to have resolved the apparent inconsistencies between previously determined cryoscopic values and those obtained by other methods.

#### Acknowledgment

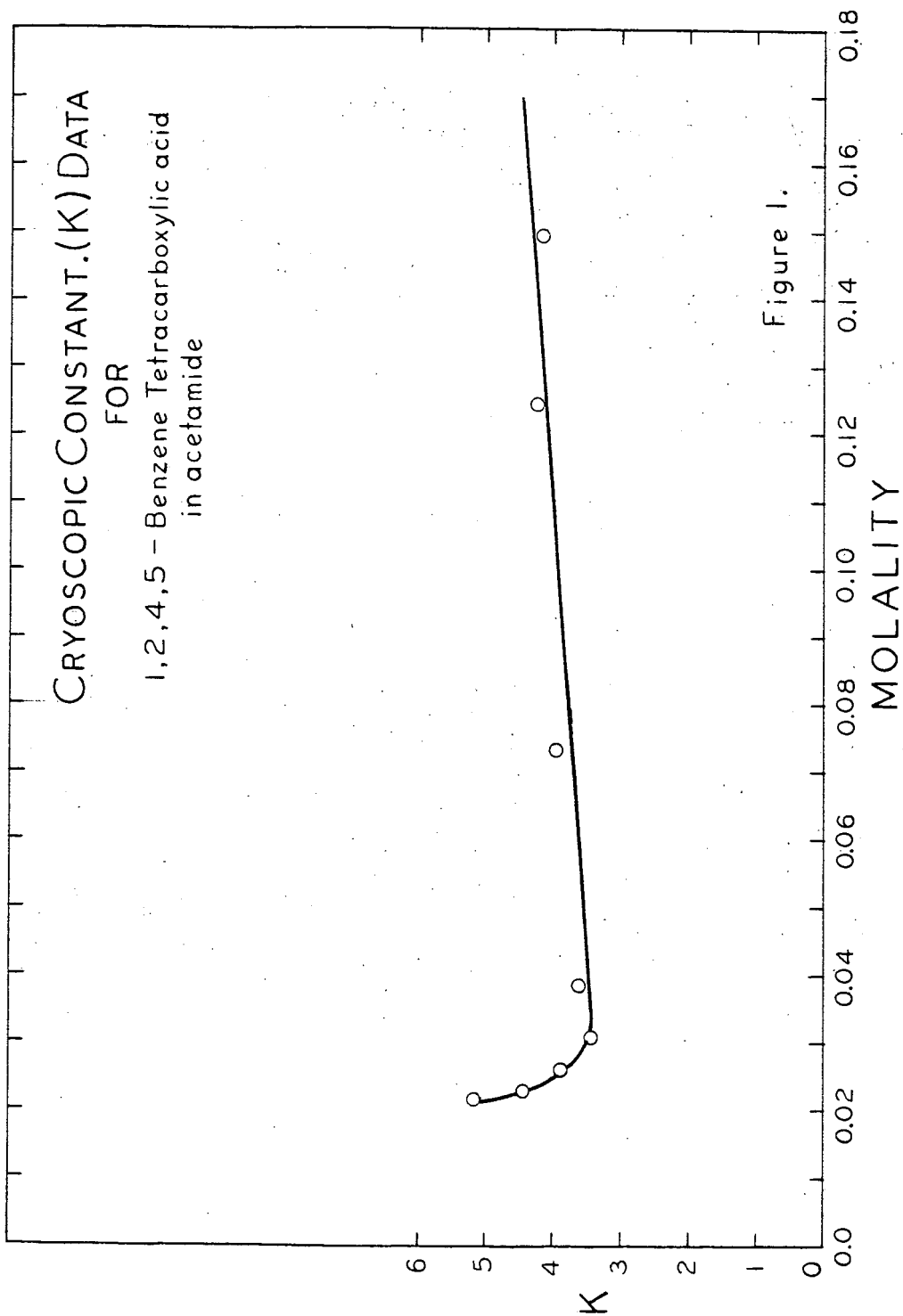
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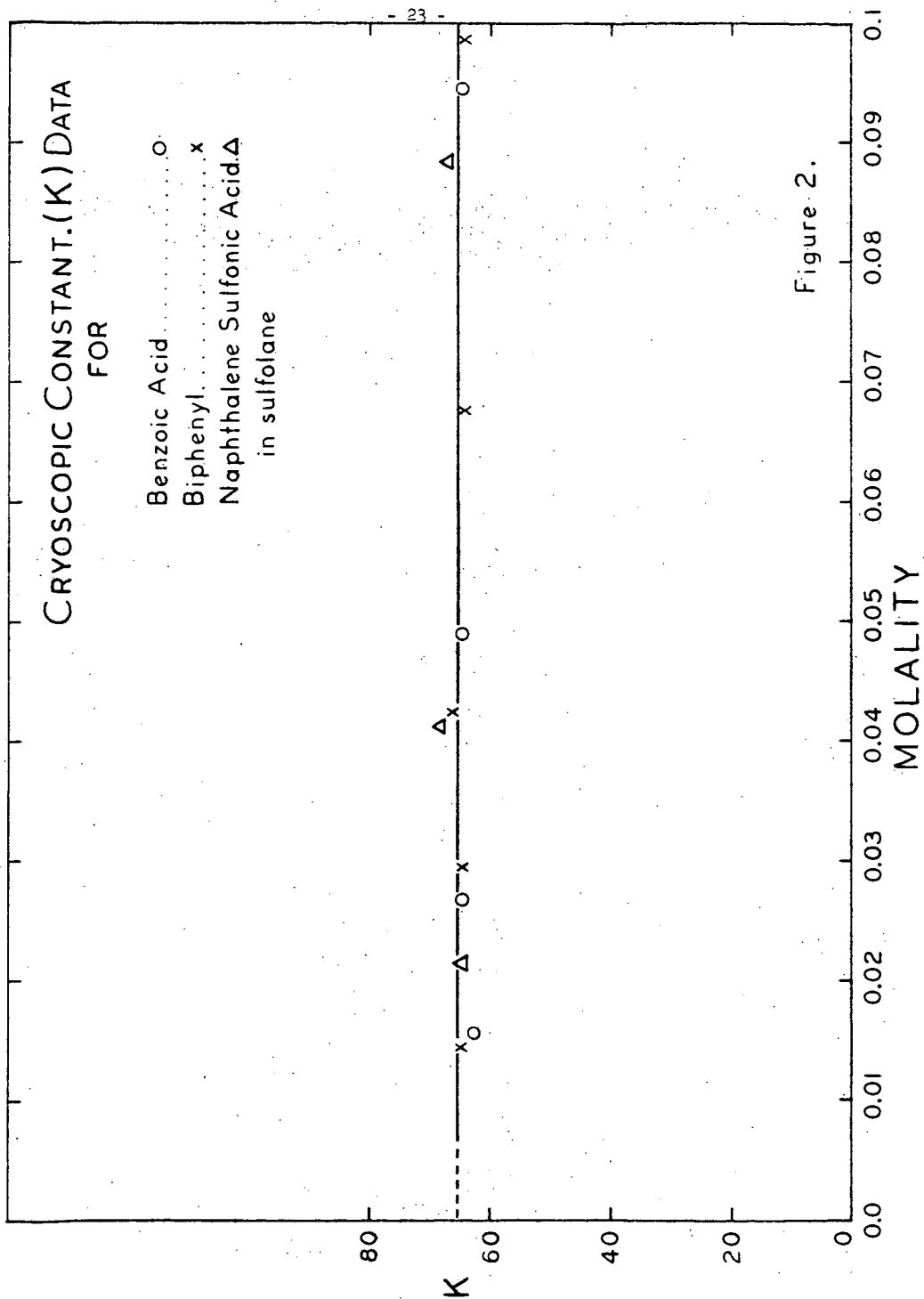
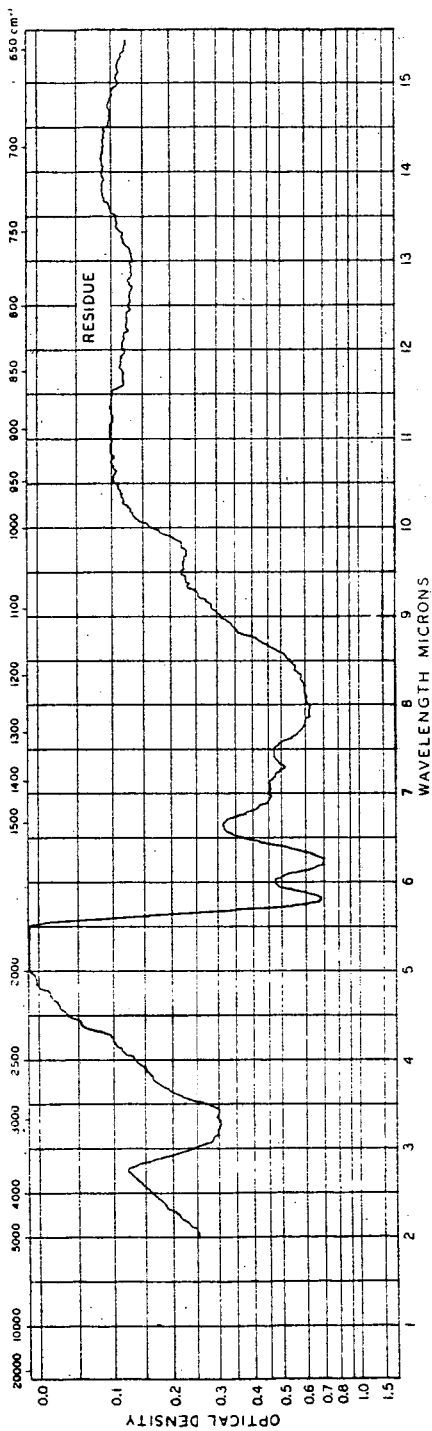


Figure 3.



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